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Chlorophylls and carotenoids of kiwifruit puree are affected similarly or less by microwave than by conventional heat processing and storage

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The impact of microwave (1000 W – 340 s) and conventional heat (97 °C – 30 s) pasteurisation and storage (4, 10, 22 °C for up to 63 d) on total and individual carotenoids and chlorophylls in kiwifruit puree was evaluated. Bioaccessibility of carotenoids, before and after pasteurisation and storage, was also studied. Microwaves and conventional heating led to marked changes in the chlorophyll (42–100% losses) and carotenoid (62–91% losses) content. First- and second-order kinetics appropriately explained the degradation of total carotenoids and chlorophylls over time, respectively. Pasteurised samples showed significantly ($p < 0.05$) enhanced stability of these pigments, with microwaves ($k = 0.007$–0.031 100 g mg^{-1} day^{-1} at 4–22 °C) promoting chlorophyll stability to a greater extent than conventional heating ($k = 0.0015$–0.034 100 g mg^{-1} day^{-1} at 4–22 °C). Bioaccessibility of carotenoids remained ($p < 0.05$) unaffected by processing and storage. These results highlighted that the pigment composition of microwaved kiwifruit was more similar to that of the fresh fruit and better preserved during storage.
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1. Introduction

Fruits have been natural components of the human diet throughout history. Although their consumption seems to have been promoted more in recent times because of their well-known nutritional value and additional associated health benefits such as chronic disease prevention (Antunes, Dandlen, Cavaco, & Miguel, 2010), they have traditionally been perceived as appetising food products, given their wide variety of inviting colours and flavours, mostly conveyed by their pigment composition (Khoo, Prasad, Kong, Jiang, & Ismail, 2011).

In the particular case of kiwifruit (*Actinidia deliciosa*), a comparatively low-calorie (57 kcal/100 g), nutritious fruit rich in vitamin C, potassium, folate and fibre (Drummond, 2013), chlorophylls and carotenoids are the main pigments that contribute to the characteristic bright green colour of its flesh (Nishiyama, Fukuda, & Oota, 2005). The potential beneficial health properties of carotenoids, in particular, such as anti-inflammatory and anti-oxidant effects (Kaulmann & Bohn, 2014; Khoo et al., 2011), have been widely recognised and have long been considered an interesting study target. Although most investigations have traditionally focused on evaluating food carotenoid content, it should be kept in mind that the positive effect of these secondary plant compounds or any other functional compounds depends not only on their content but also on the extent to which they are bioaccessible and available for absorption after ingestion and digestion (Biehler, Hoffmann, Krause, & Bohn, 2011).

On the other hand, although kiwifruit has been reported to possess great potential for industrial exploitation (Barboni, Cannac, & Chiaramonti, 2010), few processed kiwifruit products are available on the international market nowadays. During processing and storage, dramatic changes are often observed in the pigment pattern of this fruit, resulting in degradation of chlorophylls into pheophytins, pyropheophytins, chlorophyllides and pheophorbides (Cano & Marín, 1992), and *cis–trans* isomerisation of carotenoids and formation of epoxides, furanoids and other degradation products of these compounds (Khoo et al., 2011). Consequently, the typical bright green colour turns to a yellowish-brown tone (Cano & Marín, 1992), and a product with an appearance very different from that of the raw kiwifruit is obtained (Cano, 1991). Given that colour is a highly important attribute in fruit quality assessment and has a considerable influence on consumer acceptance, these undesirable changes in pigment patterns of processed kiwifruit products may represent an important limitation for their marketing.

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Consequently, development and applicability studies on different processing technologies that can guarantee safety and stability while offering superior quality foods may be the key to minimising the aforementioned potential problems, and to addressing consumer expectations regarding the increased demand for ready-to-eat foods with fresh-like characteristics (Picouet, Landl, Abadias, Castellari, & Viñas, 2009). In this respect, microwave heating is considered an interesting alternative to conventional heating methods to extend fruit shelf-life. Given the particular way in which heating takes place during microwave processing, when compared to conventional thermal treatments, microwaves lead to a faster heating rate, approaching the benefits of high-temperature, short-time processing, reducing thermal degradation and maintaining the sensory, nutritional and functional properties of the product (De Ancos, Cano, Hernández, & Monreal, 1999).

In order to investigate pigment behaviour following pasteurisation and storage of a ready-to-eat kiwifruit puree, the objectives of the present research were (i) to evaluate the effect of applying a microwave heating process on carotenoid and chlorophyll pigments of kiwifruit puree compared with a conventional heat treatment, (ii) to study the stability of these pigments during subsequent storage of the product, and (iii) to assess the impact of both heat processing and storage on the bioaccessibility of carotenoids.

2. Materials and methods

2.1. Chemicals and standards

Unless otherwise stated, all chemicals employed were of analytical or superior quality. Carotenoid standards (lutein, β-carotene, 96% purity) were purchased from CaroteNature (Lupsingen, Switzerland). All other chemicals were from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Kiwifruit preparation and processing

Eight kg of kiwifruit (*A. deliciosa* var. Hayward) was purchased from a local supermarket in Spain (Mercadona S.A., Valencia, Spain) in June 2013. Fruit pieces selected on the basis of a similar soluble solids content (13–15 °Brix) were peeled with a knife, washed with distilled water (50 mL per fruit), cut into slices ca. 10 mm thick and homogenised with a Thermomix (TM 21, Vorwerk, Spain) using the fourth power level for one minute.

The kiwifruit puree obtained was aliquoted, kept below 4 °C in darkness, and then rapidly (5 min) pasteurised by means of microwave technology and conventional heating as described below. Processing conditions were chosen on the basis of preliminary experiments to simulate equivalent pasteurisation treatments in terms of the degree of enzyme and microbial inactivation they achieved (Benlloch-Tinoco, Igual, Rodrigo, & Martínez-Navarrete, 2015).

2.2.1. Microwave treatment

A microwave oven (3038GC, NORM, China) provided with a glass turntable plate was used to treat the kiwifruit puree. A sample weighing 500 g was tempered to an initial temperature of 25 °C in a thermostatic water bath (Precisterm, Selecta, Spain) set at 30 °C for 3 min and then heated in the microwave oven in a standard-size glass beaker (9 cm inner diameter and 12 cm height) (BKL3-1K0-006O, Labbox, Barcelona, Spain) at 1000 W for 340 s. The temperature of the sample in the coldest and hottest spots, previously identified (data not shown), was continuously recorded by means of a fibre-optic probe (CR/JP/11/11671, Optcom, Dresden, Germany) which was connected to a temperature datalogger (FOTEMP1-OEM, Optcom). The treated samples, termed MW, showed a final temperature of 72 and 94 °C in the coldest and the hottest spot, respectively. They were immediately cooled in ice-water for 3 min until the puree reached 35 °C, before they were further aliquoted.

2.2.2. Conventional thermal treatment

The conventional thermal treatment consisted of heating the sample to 97 °C for 30 s in a circulating thermostatic water bath (Precisterm, Selecta). After the kiwifruit had been mashed, 20 g of puree was placed in TDT stainless steel tubes (1.3 cm inner diameter and 15 cm length) and closed with a screw stopper. A thermocouple, connected to a datalogger, was inserted through the sealed screw top in order to record the time–temperature history of the sample during the treatment. Prior to this heating step, the samples were preheated to 25 °C in a thermostatic water bath (Precisterm, Selecta) (30 °C for 30 s) to shorten and standardize the come-up time (150 s). The treated samples, termed C, were immediately cooled in ice-water for 45 s until the puree reached 35 °C, before further aliquoting.

2.3. Storage study

The heat-treated (MW, C) and the non-treated (F) kiwifruit purees were packaged into clean, sterile plastic tubes (1.7 cm inner diameter and 11.8 cm length) (Ref. 525-0153, VWR, Spain) and then stored in darkness in heat-adjustable incubators at 4, 10 and 22 °C for 7, 14, 21, 35 and 63 days. The purpose of the storage at 10 and 22 °C was to observe the changes that may take place in the samples in the case of a partial, or total, rupture of the cold chain, respectively, during the shelf-life of the product. Following the storage trials, all samples were stored at −80 °C until analysis.

2.4. Analytical procedure

The MW and C samples as well as the F samples, which were used as a control, were analysed in triplicate as described below, at day 0 and at regular time intervals for each storage temperature tested. Bioaccessibility of carotenoids in the F, MW and C purees was evaluated in triplicate at day 0 and after 63 days of storage at 10 °C as described below. Additionally, a physico-chemical characterisation of F, MW and C purees at day 0 was carried out as described below. Analyses were run in triplicate.

2.4.1. Physico-chemical properties

Water content (x_w) was measured by drying the sample to constant weight at 60 °C in a vacuum oven (Vaciotem, J.P. Selecta, Barcelona, Spain) following the AOAC (2000). Soluble solids were determined by measuring °Brix in a previously homogenised sample with a portable digital refractometer (Refracto 3PX, Mettler Toledo, Buchs, Switzerland) at 20 °C and pH using a digital pH-meter (Basic 2, Crison, Barcelona, Spain).

2.4.2. Extraction of pigments

2.4.2.1. Chemical extraction. Chlorophylls and carotenoids were extracted from the kiwifruit puree as described by Biehler, Mayer, Hoffmann, Krause, and Bohn (2010), with some modifications. In brief, 4 g of frozen kiwifruit was weighed into a 15-mL centrifuge tube (BD Biosciences, San Jose, CA, USA) and 6 mL of methanol was added together with 0.25 g of sodium carbonate to prevent rapid conversion of chlorophylls to the corresponding pheophytins. After mixing, sonication and incubation for 5 min on ice, samples were centrifuged (Harrier 18/80 refrigerated centrifuge, MSE, London, UK) for 5 min at 2500g at 4 °C. The supernatant was decanted into a 50-mL centrifuge tube, extraction was performed twice with 9 mL of a mixture of hexane and acetone (1:1, v/v) and the organic fractions were combined. Ten milliliter of

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